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Washington, D.C. 20231,
on

Feburary 5, 1997

TOWNSEND and TOWNSEND and CREW LLP

By _Linda Warren_

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:	)
	)
PETER A. SEUBERT et al.	) Examiner: Patricia Duffy
	)
Application No.: 08/419,008	) Art Unit: 1818
	)
Filed: April 7, 1995	) DECLARATION PURSUANT TO 37
	) C.F.R. § 1.131
For: METHODS FOR AIDING IN THE	)
DIAGNOSIS OF ALZHEIMER'S	)
DISEASE BY MEASURING	)
AMYLOID-β PEPTIDE (x-≥41)	)
AND TAU	)
	)

Assistant Commissioner for Patents
Washington, D.C.   20231

Sir:

        We, PETER A. SEUBERT, CARMEN VIGO-PELFREY, DALE B.

SHENK and ROBIN BARBOUR declare:

        1.   We are the named co-inventors of the inventions

claimed in the above-captioned application. We make this

declaration to present facts establishing that methods of

measuring soluble Aβ(x-≥41) described in the above-captioned

application were invented before January 1994. All the work

described hereinbelow was performed in the United States of

America.

        2.   Attached hereto at Exhibit 1 are copies of

laboratory notebook pages numbered 52-55 and 58 kept by Carmen

Vigo-Pelfrey. Carmen Vigo-Pelfrey signed these pages on the

bottom left-hand side using her maiden name, "C. Vigo." The

dates on these notebook pages indicated by Carmen Vigo-Pelfrey

is actually $A\beta_{33-42}$ with cysteine amino-heptanoic acid added at the amino terminus for purposes of conjugation to a carrier peptide.

5.   In the sandwich assay described in the preceding paragraph, Carmen Vigo-Pelfrey stated that the second antibody was "277-2." Antibody 277-2 is specific for $A\beta_{1-42}$. (See also the specification, page 27, section b.)

6.   Carmen Vigo-Pelfrey did not identify the capture antibody, stating only that "Plates were coated with 5 μg/ml and fixed with 0.25% HSA." However, the capture antibody is identified on page 58 of the notebook. That page refers to the detection of $A\beta_{1-42}$. There, in the middle of the page, Carmen Vigo-Pelfrey stated, "The assay was performed as described in p 52." Above that statement, the Plate Map section indicates the antibodies used: "266-272-2". "266" is a reference to the capture antibody. Antibody 266 is specific for the junction region of $A\beta$. (See also the specification on pages 25-27.)

7.   The reference to "272-2" is, in our opinion, an erroneous reference to antibody "277-2". We are familiar with all of the antibodies used in these experiments conducted at Athena Neurosciences. We did not have any antibodies called "272-2".

8.   Carmen Vigo-Pelfrey showed that the sandwich assay described could detect $A\beta_{1-42}$ and distinguish it from $A\beta_{1-28}$, $A\beta_{1-38}$ and $A\beta_{1-40}$. Pages 53-55 of the notebook show data produced using the above assay to generate standard curves for $A\beta_{1-42}$, $A\beta_{1-28}$, $A\beta_{1-38}$ and $A\beta_{1-40}$. The standard curve on page 55 shows the specific detection of $A\beta_{1-42}$ in the assay. Below the standard curve on page 55 Carmen Vigo-Pelfrey stated, "This assay detects $A\beta_{1-42}$ immunoreactivity with sensitivity greater than 0.625 and with no cross reactivity with $A\beta_{1-40}$, $_{1-38}$ or $A\beta_{1-28}$."

9.   Thus, the notebook pages show the actual detection of $A\beta_{1-42}$ in a sandwich ELISA in which antibody 266, directed to

the junction region of Aβ, was used as the capture binding
substance and antibody 277-2, directed to the carboxy terminus of
Aβ₁₋₄₂, was used as the detection binding substance. In these
experiments, the amount of binding by the detection binding
substance was determined using a reporter antibody, which was an
enzymatically labeled antibody specific for rabbit antibodies.

10. We hereby declare that all statements made herein
of our own knowledge are true, and that all statements made on
information and belief are believed to be true; and, further,
that these statements were made with the knowledge that willful
false statements and the like so made are punishable by fine or
imprisonment, or both, under Section 1001 of Title 18 of the
United States Code, and that such willful false statements may
jeopardize the validity of the application of any patent issued
thereon.

Date: January 28, 1997   Peter A. Seubert

Date: January 24, 1997   Carmen Vigo-Pelfrey

Date: January 29, 1997   Dale B. Schenk
                                               Schenk

Date: January 30, 1997   Robin Barbour

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Development of Aβ 1-42 assay

An assay to measure Aβ 1-42
has been developed

Plates were coated with 5H5/ul and
fixed with 0.25% HSA

Samples/calibrators 1-42 0.125 - 10ng/ml
were added, 100 ul/well and incubated
for 1h at RT

The super was aspirated, plates washed
3x and alkaline 277-2 poly rabbit
polyclonal antibody, raised against
the last 10 amino acids of Aβ
32-42, added at 1ng/ml in
conjugate diluent, 100 ul/well, and
incubated at RT, for 1h

Fluid is aspirated and washed 3x.
Alkaline phosphatase conjugated
donkey X(rabbit)F mouse adsorbed
diluted 1:1000 in conjugate diluent
was added 100 ul/well and incubated
for 1h at RT

Sup. aspirated, plates washed 3x

Fluorescent substrate MUP added 100ul/w
and incubated for 1h at RT 15 min at
RT. Plates read

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l-V.P̌o   REDACTED   S.Chugȟ   MAR 10 1994
Signed   Date   Signed   Date

Cross reactivity of Aβ₁-₄₂ with Aβ₁-₄₀, ₁-₃₈ and
Aβ₁-₂₈ was studied. The effect of Triton NP40
and octalglucoside NP40 and SDS and boiling
was studied.

Plate Map)   ▲ athena neurosciences

	1	2	3	4	5	6	7	8	9	10	11	12
A	0	625	1	25	2	5	5	0		10	0	B
B												
C												
D												
E												
F												
G												
H												

← Aβ₁-₄₂
← Aβ₁-₄₀
← Aβ₁-₃₈
← Aβ₁-₂₈
{ same + 1% triton

Plate #2 Same as 1 but Rows E-F with
(CV? 2% triton X 100) 0.2% NP40.

Plate #3 Same as 1 , Rows E-F Aβ₁-₄₂ boiled

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PROJECT __A β₁-42__

	1	2	3	4	5	6	7	8	9	10	11	12
A Set 1	896	742	1386	1390	1867	1820	2089	2037	2167	2161	158	175
B Set 1	105	98	98	156	153	111	187	139	221	165	199	223
C Set 1	98	100	83	110	103	121	113	95	97	97	99	108
D Set 1	97	97	84	121	113	122	135	100	95	105	114	107
E Set 1	381	302	604	540	1052	945	1466	1474	1825	1841	103	111
F Set 1	116	93	93	124	108	126	144	91	99	111	110	136
G Set 1	122	126	203	121	114	124	179	124	93	102	162	145
H Set 1	77	73	77	101	128	123	103	108	100	100	136	161

plate #2

	1	2	3	4	5	6	7	8	9	10	11	12
A Set 1	684	636	1119	1174	1682	1654	1958	1964	2083	2119	117	110
B Set 1	135	146	111	139	124	131	147	119	120	135	120	139
C Set 1	123	108	98	98	120	115	120	122	134	125	115	125
D Set 1	102	89	90	92	92	89	106	102	89	100	107	118
E Set 1	378	222	455	502	940	1006	1397	1366	1564	1586	158	231
F Set 1	88	93	87	108	112	87	97	96	90	97	129	120
G Set 1	125	114	93	138	150	129	174	185	111	116	125	109
H Set 1	64	61	60	64	68	119	121	83	82	83	121	143

plate #3

	1	2	3	4	5	6	7	8	9	10	11	12
A Set 1	1085	889	914	1049	1508	1617	2031	1904	2210	2204	232	207
B Set 1	256	222	188	176	176	171	255	257	198	246	247	218
C Set 1	260	191	309	355	322	304	346	270	309	305	261	281
D Set 1	176	165	171	162	174	205	279	221	213	195	232	239
E Set 1	383	368	562	622	1110	922	1678	1631	1958	1986	210	218
F Set 1	202	252	196	221	255	245	280	316	332	346	209	246
G Set 1	250	203	132	169	210	229	279	261	277	337	230	351
H Set 1	213	186	194	217	224	271	278	299	285	311	205	205

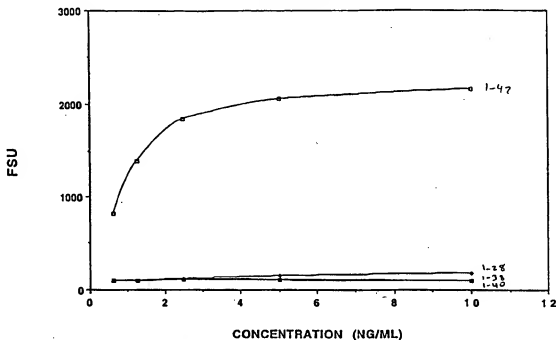Read and Understood By

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Signed

REDACTED
Date

____Signed____

PROJECT ___AB_1-42___

AB 1-42, 1-40,1-38,1-28 STD CURVE (266-272)

This assay detects AB-42 immunoreactivity with sensitivity greater than 0.625 and with no cross reactivity with AB-40,-38 or AB-28

There is little effect of triton X-100, NP-40 or boiled. This can have relevant consequences in sample assay, CSF in particle

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Signed   Date   Sign d   Date

Effect of Triton X-100 NP-40 and boiled
on AB 1-42 Std. curve and two CSF
samples

▲ athena neurosciences

Plate Map

266 - 272 - 2   7 | 1 | 7/93

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.125	0.625	1.25	2.5	5.0		AB ←
B							← Boiled
C							← T
D							← NP40
E	1212 Neat	1212 Boiled	12 2 T	1212 NP40	1406 Neat	1406 Boiled
F	1406 T	1406 NP40	AB Cell	AB Cell	AB
G	CSF	NM			
H					

The assay was performed as described in p 52

		1	2	3	4	5	6	7	8	9	10	11	12
A	Set 1	217	203	997	1009	1604	1582	1997	1958	2161	2167	90	83
B	Set 1	173	158	417	489	838	914	1503	1433	1820	1920	102	90
C	Set 1	154	141	407	432	833	842	1425	1235	1721	1750	112	109
D	Set 1	139	145	456	530	997	1049	1516	1314	1915	1936	107	109
E	Set 1	350	364	424	394	167	190	216	215	740	746	978	994
F	Set 1	332	296	294	278	124	116	120	114	105	97	91	95
G	Set 1	282	279	141	139	150	142	134	132	128	117	117	117
H	Set 1	126	128	127	127	132	127	123	126	118	110	114	133

MAR 10 1994

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e. U f o   REDACTED   S. Chugler
   Signed   Date   Signed   Date